

REMARKS

Responsive to the requirement for submission of a Sequence Listing, imposed in the outstanding Official Action, the same is provided herewith, attached to the present amendment, in paper and disk formats. Applicants hereby state that the attached paper and computer-readable copies have the same content, and introduce no new matter into the present application.

In this regard, the specification has been amended so that it is commensurate with the submission of the present Sequence Listing. It is respectfully submitted that no new matter has been added.

The outstanding Official Action also required that an oath or declaration of the inventors be submitted. In response to this requirement, we also submit a Combined Declaration and Power of Attorney from the inventors.

It is respectfully submitted that the above-identified application complies with the requirements for patent applications containing nucleotide sequence and/or amino acid sequence disclosures.

Favorable action on the merits of the present application, in view of the above, is now respectfully requested.

GOLAY et al. S.N. 12/193,901

Attached hereto is a marked-up version of the changes made to the specification. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully submitted,

YOUNG & THOMPSON

By *Philip A. DuBois*  
Philip A. DuBois  
Agent for Applicants  
Registration No. 50,696  
745 South 23rd Street  
Arlington, VA 22202  
Telephone: 521-2297

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VERSION WITH MARKINGS TO SHOW CHANGES MADE  
IN THE SPECIFICATION:

Paragraph beginning at line 20 of page 5 has been amended as follows:

For the amplification, 40 ng of plasmid were brought in a final reaction volume of 100 µl in 10 mM KCl, 10 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 20 mM Tris HCl, pH 8.75, 2 mM MgSO<sub>4</sub>, 0.1% Triton X-100, 100 µg BSA, in the presence of 0.8 µl of a solution of 2.5 mM dNTP, 500 ng of primer "sense" (CGGGATCCAAAATGACAACACCCAGAAATTC (SEQ Id. No. 1)), 500 ng of primer "antisense" (CGGGATCCTTAAGGAGAGCTTCATTTTCT (SEQ Id. No. 2)) and 50 Pfu DNA Polymerase from Strategene (La Jolla, CA, USA). The reaction was carried out for 25 cycles in the cyclor following this scheme: 1' at 95°C, 1' at 60°C and 2' at 72°C. At the end of the reaction 100 µl of a 25:24:1 phenol chloroform and isoamyl alcohol solution were added and after extraction, DNA was precipitated overnight at 20°C in the presence of ethanol. After centrifugation, DNA was resuspended in 100 µl water and then subcloned in the pMOS vector (Amersham Italia, srl, Italy) according to the manufacturer's instructions contained in the kit "pMOS blunt end cloning kit". The resulting recombinant plasmid was amplified and sequenced, then digested with BamHI whose recognition site (G/GATCC) was present in both PCR primers' ends. Therefore the fragment was subcloned in the BamHI site of the retroviral vector PINCO VUOTO. The retroviral vector PINCO VUOTO

had been previously obtained following excision with EcoRI and NotI of a 1441 bp fragment containing the CMV promoter (Cytomegalovirus) and the EGFP (enhanced green fluorescent protein) gene from the plasmid PINCO (F.Grignani et al., Cancer Res., 58, 14-19, 1998). After excision of the EcoRI-NotI fragment, the plasmid was closed after end blunting with Klenow fragment and called VINCO VUOTO. Such retroviral vector is now of 11448 bp in length.